DEVICE AND METHOD FOR DETECTION AND IDENTIFICATION OF BIOLOGICAL AGENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and incorporates by reference in its entirety United States Provisional Patent Application No. 60/406,665 entitled "METHOD AND APPARAUS FOR DETECTION AND ANALYSIS" filed August 29, 2002.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The present invention relates generally to a sensor system, and more specifically to a sensor for detecting biological and chemical agents in the environment.

Description of the Related Art

[0003] Antibody-based detection systems are the most mature and advanced technology for biological agent detection and identification. Antibodies are defined as any of the body immunoglobulins that are produced in response to specific antigens and that counteract their effects by neutralizing toxins, agglutinating bacteria or cells, and precipitating soluble antigens. Antigens are defined as protein or carbohydrate substances capable of stimulating an immune response. Antibodies are very specific and bind only to their target, even in the presence of other material. In a detector, antibodies are normally immobilized on a substrate, e.g., a polymer, such as polyvinylethylene, polyethylene, or polystyrene, for subsequent incubation with the target organism or molecule. Typically, the antibodies are not chemically bound to the substrate, but merely attached by hydrogen bonding or electrostatic charge. The antibody and antigen bind upon contact, thereby immobilizing the antigen. Classically, a second antibody to the target agent incubates, as well as binds to the antigen. This second antibody is generally linked to some type of reporter system, usually an enzyme. The varying forms of these reporter systems include, e.g., fluorescent, magnetic, enzymatic, colorimetric, etc. This transducer provides the means of

detecting the presence of the antigen of interest. Enzyme linked immunosorbent assay (ELISA) is based on this process.

[0004] An analyte in the antibody-antigen detection system is typically in an aqueous solution or other liquid solution. The aqueous solution must make frequent and intimate contact with the immobilized antibody on the substrate material. A large surface area on the substrate allows a higher density of antibodies and hence a higher sensitivity. However, the antibodies must be tightly bound to the substrate to survive repeated motion of the analyte over the substrate without becoming detached and flushed away with the solution. Therefore, covalent bonding, rather than hydrogen bonding or electrostatic bonding, of the antibodies to the substrate is preferential. Many biological compounds of interest in the system are proteins, e.g., enzymes, hormones, toxins, antibodies, and antigens. Proteins are composed of amino acids, having both an amino group (NH_2) and a carboxylic acid group (COOH). A substrate functionalized with one or both of these groups can be activated to chemically bind antibodies.

[0005] The introduction of a second antibody in the ELISA process complicates and slows down the detection/identification process. A physical property change produced by the antibody-antigen chemical reaction provides the basis for a more direct transduction mechanism. The transduction mechanism in an optics-based detection system is based on a change in absorption or index of refraction, which is monitored by the optical system.

[0006] Several detectors are based on a change of index of refraction. One such sensor is based on surface plasmon resonance. Surface plasmon techniques are difficult to integrate for multiplexed operation where multiple target agents can be monitored simultaneously. Also, their sensitivity cannot be engineered by sharpening the spectral or angular response to light.

[0007] Other known sensors include a chemical sensor based on porous silicon and a porous-semiconductor-based, e.g., porous Si, optical interferometric sensor. Interference filters can be made with porous silicon. However, porous silicon interference filters are incompatible with polymer waveguide technology and hence cannot be readily integrated onto a polymer waveguide chip. Also, porous polymers are easier to apodize, i.e., sharpen, their spectral response using holographic techniques. Moreover, polymer chemistry is more adaptable to functionalization with chemical groups for binding antibodies and antigens. Most immunosorbent assays are

conducted on polymer substrates. Porous silicon has not been widely used for this application. No conventional methods propose the simultaneous use of porous semiconductors as both chemical and optical filters.

[0008] Another known sensor is a doubly-differential interferometer-based sensor with evanescent wave surface detection. This sensor is a surface detector only and cannot take advantage of the extended surface area of a porous polymer. Furthermore, the sensor also requires polarized light and a modulator. Additionally, this sensor is a part of a system that does not provide for continuously monitoring the environment. The interferometer is also not flexible for sharpening the optical response for higher sensitivity.

[0009] Another sensor, in the form of polymerized crystalline colloidal arrays, achieves detection of chemical and biological agents by a change in diffraction accompanying the swelling or shrinkage of a hydrogel containing the crystalline colloidal array in response to a chemical reaction with target agent(s). Similarly, a conventional hologram-containing sensor consists of a holographic grating recorded preferably in a gelatin, where reaction of chemical agents with the gelatin produces some change in the physical properties of the hologram matrix, thereby changing the diffraction properties of the hologram. In both the polymerized crystalline arrays and the hologram-containing sensor, a matrix containing a grating serves to uptake an analyte, but does not allow for the analyte to flow through the system. Once the system is swollen, the only mechanism for replacing it with new samples is to remove the grating from the system and dry it out. Since the materials used are not porous, the system cannot take advantage of increased surface area to volume ratio and does not provide a convenient method for chemically filtering the analyte. These methods are also not compatible with waveguides for integration onto a chip.

[0010] Another chemical and biochemical sensor includes a planar waveguide with a grating coupler. A recognition layer containing specific chemical or biochemical binding partners, e.g., antibodies or antigens, is located on the waveguide. A chemical reaction on the recognition layer changes the effective refractive index of the guiding layer, thereby changing the coupling efficiency of the grating, i.e., the angle of incidence for maximum input coupling to the waveguide. Using this sensor, a method for optical determination of an analyte records the position of light points with a position sensitive detection method. The grating is a surface

grating formed by standard methods, i.e., photolithographic patterning followed by etching. A surface grating sensor cannot take advantage of the extended surface area of a porous polymer, since the grating cannot be extended throughout the volume of the porous polymer and chemical detector or recognition molecules cannot be dispersed throughout the volume to increase its chemical sensitivity. This system does not provide a mechanism for continuously monitoring the environment by flowing the analyte through the grating, since it is only a surface grating. Nor does the system use the grating as an optical filter to take advantage of the sharp spectral properties of a Bragg grating for detecting large changes in transmission of such a filter with relatively small changes in refractive index.

SUMMARY OF THE INVENTION

Summary of the Problem:

[0011] The rapid detection and identification of hazardous biological and chemical agents has become an increasing concern due to the dangers of biological and chemical warfare as well as the threat of terrorists releasing such agents in public venues. Before troops are deployed in forward staging areas, biosensors need to assess the environment for force protection. In terrorist situations, adequate security measures require continuous monitoring of high value public areas, such as government buildings, subways, stadiums, water treatment plants, etc. First responders to a biological or chemical terrorist attack need to quickly and accurately detect and identify the particular biological or chemical agent to take necessary precautions and adequately administer aid. Although standoff sensors, such as lidar, provide some remote sensing of the release of agents, they cannot identify particular agents. Point detection systems can sense the immediate environment. Specificity is a necessary ingredient in the sensor system. Compact, rugged, reliable sensor systems that can continuously monitor the environment are desired. With appropriate telemetry systems, these can be deployed in forward battle areas during warfare (for example, delivered by drones or dropped by parachute) or placed in high value public areas to continuously monitor and report environmental changes that may indicate a terrorist attack.

[0012] Conventional means of detecting biological agents in the environment, e.g., laser induced fluorescence, accurately detect the presence of biological agents. These detectors do not,

however, provide the specificity to identify the agents present. Additionally, these devices are complex and bulky. Other devices capable of agent detection and identification, e.g., mass spectrometry, are expensive and not easily portable. These devices take a considerable amount of time to identify the agent(s). Still other devices, utilizing immunoassay techniques, identify agents with high specificity by antigen-antibody chemical reactions. Unfortunately, these techniques are not readily amenable to providing continuous, always-on monitoring of the environment. Moreover, these techniques are not reagentless; they normally require additional chemistry to add chromophores or fluorescing agents for detection and identification by color change or fluorescence. A need exists for a compact, inexpensive, portable biosensor system that can continuously monitor the environment, i.e., always-on mode, and both detect and identify biological agents in the environment with high specificity and a low false alarm rate.

Summary of the Solution:

[0013] The conventional methods neither achieve nor teach methods or devices to meet the above-mentioned criteria for a biosensor. Therefore, it is an object of this invention to provide a compact, inexpensive, rugged and portable biosensor that can continuously monitor the environment for detection and identification of hazardous biological agents.

[0014] The solution to the conventional methods is to continuously monitor the environment for hazardous biological and biochemical agents, providing rapid, automatic, simultaneous detection and identification of such agents with high specificity and low false alarm rate. Such a system continuously draw samples from the environment, i.e., air, water, or soil, for analysis. The system repeatedly and indefinitely exposes the detector to the samples. The detector specifically recognizes the target agents and responds by some physical or chemical change of state. Based on the change in the detector state, a transduction mechanism produces a useable signal. The detection mechanism is highly sensitive, achieving a rapid response with low probability of false signals, whether positive or negative indications. The detector is rugged and can reliably respond even after being subjected to multiple sample exposures. The system provides a platform insusceptible to external temperature swings and vibrations.

[0015] It is furthermore an object of this invention to monitor air and/or water and/or soil continuously for the detection and/or identification of hazardous biological agents.

[0016] It is furthermore an object of this invention to provide a working fluid that is continuously circulated in the sensor as a medium for transporting environmental samples to detector modules for always-on monitoring of the presence of hazardous biological agents.

[0017] It is furthermore an object of this invention to provide a detector consisting of a porous polymer Bragg grating that functions simultaneously as a chemical filter, to trap specific target agents for detection by a highly specific chemical reaction with a conjugate molecule, and as an optical filter that provides the transduction mechanism to create a measurable signal stemming from the chemical reaction.

[0018] It is furthermore an object of this invention to provide a detector consisting of a porous polymer Bragg grating that has a high surface area to volume ratio to provide a high density of binder molecules that increases the probability of target agent binding and hence increases the detection sensitivity.

[0019] It is furthermore an object of this invention to provide a detector consisting of a porous polymer Bragg grating that can be fabricated holographically as a thick filter with low index modulation, hence achieving a sharp spectral transmission or reflection notch that enhances the detection sensitivity.

[0020] It is furthermore an object of this invention to provide a detector consisting of a porous polymer Bragg grating that can be fabricated holographically to apodize the filter, sharpen the spectral response and enhance the detection sensitivity.

[0021] It is furthermore an object of this invention to provide an array of detector modules consisting of porous polymer Bragg gratings, each of which is sensitized with a different molecule for binding specific target agents and can hence monitor the presence of multiple hazardous biological agents in the environment.

[0022] It is furthermore an object of this invention to provide an array of detector modules consisting of porous polymer Bragg gratings, where each module consists of more than one detector arm having said porous polymer Bragg gratings, and only one arm is sensitized with a

particular detector molecule, the other arm(s) serving as control and reference that factor out the effects of thermal and light source fluctuations and drift, or other environmental disturbances, and factor out transient effects from inert microscopic material contained in the environmental sample, thereby achieving a low false alarm rate.

[0023] It is furthermore an object of this invention to provide a system that does not require additional chemistry to add chromophores or fluorescing agents for detection and identification by color change or fluorescence.

[0024] It is furthermore an object of this invention to provide detection sensitivity by combined optical and electronic differential gain.

[0025] It is furthermore an object of this invention to provide a sensor capable of rapid response due to high detection sensitivity.

[0026] A first embodiment of the present invention describes a method of determining a target agent in an environment comprising the steps of obtaining a first sample from the environment and introducing the first sample to at least one detection module. The first sample is then filtered through at least a first filter and a second filter comprising at least one detection module, wherein the first filter contains at least one detection molecule for the target agent and the second filter contains no detection molecules for the target agent. An optical property is measured from the first filter and the second filter after filtering the first sample there through. Comparing the measured optical property of the first filter to the measured optical property of the second filter assists in determining the presence of the target agent.

[0027] A second embodiment of the present invention describes a sensor for determining the presence of at least one target agent in a sample comprising a collector system for collecting the sample from an environment, a transfer system for adding the sample to a working fluid, a dispenser system for introducing the working fluid, including the sample, to a detector system, and a detector system comprising at least one detector module. The detector module includes at least a first optical grating and a second optical grating, wherein the first optical grating contains at least one detector molecule for detecting the at least one target agent and the second optical grating does not contain a detector molecule for detecting the at least one target agent. The

detector module further includes at least a first measuring device for measuring an optical response of the first optical grating after contact with the working fluid, including the sample, and at least a second measuring device for measuring an optical response of the second optical grating after contact with the working fluid, including the sample. A processor compares the measured optical response from the at least a first measuring device to the measured optical response from the at least a second measuring device to determine the presence of the target agent.

[0028] A third embodiment of the present invention describes a detector module for detecting a target agent within a sample comprising at least one inlet reservoir for receiving the sample therein, a first micro-fluidic channel integrally connected to the at least one inlet reservoir, a second micro-fluidic channel integrally connected to the at least one inlet reservoir, a first optical grating physically integrated with the first micro-fluidic channel and a second optical grating physically integrated with the second micro-fluidic channel, wherein the first optical grating includes at least one detector molecule for detecting the target agent within the sample and the second optical grating does not include a detector molecule for detecting the target agent within the sample. The detector module also comprises at least one outlet reservoir physically integrated with the first micro-fluidic channel for removing the sample from the detector module.

[0029] A fourth embodiment of the present invention describes a method for forming an optical sensor for sensing the presence of a target agent in a sample comprising interfering a first coherent beam and a second coherent beam within a polymerizable polymer-dispersed liquid crystal material forming a polymerized hologram containing liquid crystals within a polymer matrix. The liquid crystals are extracted from the polymer matrix forming pores therein. The binding sites within the pores are chemically activated for receipt of a detector molecule therein. A detector molecule is attached within the pores for sensing the presence of a target agent in a sample.

BRIEF DESCRIPTION OF THE FIGURES

[0030] The present invention will be more clearly understood from a reading of the following description in conjunction with the accompanying figures wherein:

[0031] **Figure 1** shows a detection system according to an embodiment of the present invention;

[0032] **Figure 2** shows a subsystem of a detection system according to an embodiment of the present invention;

[0033] **Figure 3** shows a detection/identification subsystem of a detection system according to an embodiment of the present invention;

[0034] **Figure 4** shows a waveguide circuit according to an embodiment of the present invention;

[0035] **Figure 5** shows a waveguide circuit according to an embodiment of the present invention;

[0036] **Figures 6a** and **6b** show electronic processing according to an embodiment of the present invention;

[0037] **Figure 7** shows an embodiment with dual reference arms according to the present invention;

[0038] **Figures 8a** and **8b** show a grating in a waveguide according to an embodiment of the present invention;

[0039] **Figure 9** shows a corrugated waveguide with a porous polymer cladding according to an embodiment of the present invention;

[0040] **Figure 10** shows a grating in cladding according to an embodiment of the present invention;

[0041] **Figure 11** shows channel waveguides according to an embodiment of the present invention;

[0042] **Figure 12** shows a grating in a waveguide according to an embodiment of the present invention;

[0043] **Figure 13** shows a waveguide according to an embodiment of the present invention;

[0044] **Figures 14a-14c** show a fabrication process for gratings according to an embodiment of the present invention;

[0045] **Figure 15** shows a spectral diffraction efficiency according to an embodiment of the present invention;

[0046] **Figure 16** shows a spectral diffraction efficiency according to an embodiment of the present invention;

[0047] **Figure 17** shows a spectral diffraction efficiency according to an embodiment of the present invention;

[0048] **Figures 18a-18c** show a process for obtaining an intensity distribution according to an embodiment of the present invention;

[0049] **Figure 19** shows transmission notches for different refractive indices according to an embodiment of the present invention;

[0050] **Figure 20** shows a spectral diffraction efficiency according to an embodiment of the present invention;

[0051] **Figures 21a** and **21b** show a spectral diffraction efficiency according to an embodiment of the present invention;

[0052] **Figure 22** shows transmission scans according to an embodiment of the present invention;

[0053] **Figure 23** shows a transmission scan according to an embodiment of the present invention; **Figure 24** shows a relation of absorbance to a concentration of antigen according to an embodiment of the present invention; and

[0054] **Figure 25** shows a grating situated in a waveguide according to an embodiment of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE PRESENT INVENTION

[0055] In an embodiment of the present invention as shown in **Fig. 1**, a biological and chemical target agent sensor **10** has a sample collection subsystem **20** that continuously draws samples of air, water, and soil from the environment. A transfer subsystem **30** transfers the sample **40** from the collection subsystem **20** to working fluid **50**. A dispenser subsystem **60** dispenses the working fluid **50** containing the sample into a detector module array ("DMA") of a detection/identification (hereafter "ID") subsystem **70**.

[0056] The dual-function ID subsystem **70** sends the sample data from the DMA through an electronic signal **80** to a data storage subsystem **90**. Data storage subsystem **90** obtains and continuously compiles data from the DMA. The data within data storage subsystem **90** may either be accessed directly or may be sent via an appropriate transmission system, such as telemetry subsystem **110**, for recording and analysis. The transmission system may send data at pre-selected intervals in batch format or, alternatively, may send data on a continual basis. Further still, the data storage system **90** may perform analysis on the data from the ID subsystem **70** prior to forwarding for transmission. With this embodiment, the data storage subsystem **90** may be programmed to transmit data when a particular result is found, e.g., a target agent has been identified.

[0057] As a second function, after passing through the ID subsystem **70**, the working fluid **50** is transferred to a recirculation subsystem **120**. Recirculation subsystem **120** decontaminates the working fluid **50** and returns the working fluid **50** to the transfer subsystem **30** to obtain another environmental sample. The recirculation subsystem **120** consists of an ultrafine filter and an ultraviolet lamp that destroys/removes foreign material remaining in the working fluid. The working fluid is passed through micro-tubing and a low-flow pump. The working fluid then returns to the transfer subsystem **30** to pick up another sample **40** of the environment. The constant sampling and recirculation allows the system to continuously monitor the environment. In essence, it is "always on."

[0058] At the onset of the system of sensor **10**, collection subsystem **20** has an air sampler, preferably consisting of a pump for drawing through air from the environment, a horn or similar instrument for directing the air flow, a filter stage consisting of one or more filters to remove large particles, e.g., greater than 10 μm, and an exhaust system. For sampling gases and aerosols in the air, the collection subsystem **20** is adapted from conventional air samplers such as those provided by Airmetrics, Mattson-Garvin, SKC, or Sceptor Industries. For sampling water, e.g., standing water, wastewater, etc., the collection subsystem **20** is adapted from conventional water samplers such as the Markland Duckbill® Sampling System, or an equivalent supplied by such companies as Global Water Instrumentation, Inc., and others. In one embodiment, the water sampler consists of a remote sampler head submerged in the water or a tube submerged in the water. A sampler pump/controller draws water samples through a filter stage to remove large particles, e.g., greater than 10 μm. The water sample is then retained in a holding container for transfer of microscopic agents to the working fluid. The water is then flushed out of the system to make room for the next sample. For sampling soil, the collection subsystem **20** is adapted from conventional soil water samplers such as those supplied by Soil Monitoring Engineering. Soil water samplers, such as lysimeters or porous ceramic cups, are buried in the soil. A pump creates a partial vacuum, which causes water in the surrounding soil to enter the sampler via a porous ceramic filter. The sample is then retained in a holding container for transfer of microscopic agents to the working fluid. The water is then flushed out of the system to make room for the next sample.

[0059] The environmental samples are transferred to a working fluid by a transfer subsystem **30**. The transfer subsystem **30** transfers potentially hazardous microscopic agents, as well as other microscopic constituents, from the environment to working fluid **50** of the sensor **10**. Transfer subsystem **30** may be adapted from conventional products. For gas/water transfers, the transfer subsystem **30** is adapted from conventional gas bubblers, such as those supplied by SKC. The air sample is bubbled through the working fluid, and gases from the atmosphere are dissolved in the fluid. For aerosol/water transfers, the transfer subsystem **30** is adapted from conventional particle impingers, such as those supplied by SKC. Aerosol particles impinge on the working fluid and become trapped in the liquid. For water/water transfers, the transfer

subsystem **30** is adapted from conventional dialysis cells, such as the DMLS™ supplied by Margan. The gradient of concentrations allows for material dissolved in the environmental water to diffuse into the working fluid **50** contained in the dialysis cell.

[0060] The working fluid **50** is typically an aqueous solution compatible with the molecular detectors. The working fluid **50** continuously flows through the system to provide an always-on monitoring device. Although the working fluid **50** necessarily comes into contact with the environment, it must be held at a constant temperature and appropriate chemical composition, e.g., pH, to optimize the sensor **10** and enhance the lifetime of the complex detector molecules, e.g., antibody proteins. A heater or a thermoelectric cooler system controls the temperature of the working fluid for optimum operation of about 30-40 °C.

[0061] Dispenser subsystem **60** receives the working fluid from transfer subsystem **30**. Referring to **Fig. 1**, dispenser subsystem **60** dispenses working fluid **50**, containing the microscopic environmental samples, to ID subsystem **70** comprising a multi-channel DMA. Further to dispenser subsystem **60**, a mixer mechanically agitates the working fluid **50** to form a homogenous mixture of agents throughout the volume of the working fluid **50**. This ensures that when the working fluid passes from the dispenser **60** to the ID subsystem **70**, each channel of the DMA monitors identical samples. After passing through a filter to separate out larger particles (e.g., greater than 1 μm or greater than 0.1 μm, depending on filter selection), the working fluid **50** then passes to an array of micro-wells. Each micro-well of the micro-well array is capable of holding approximately 1 μL of working fluid. Referring to **Fig. 2**, from each of the micro-wells **200**, one of an array of syringes **240** draws a carefully metered volume of working fluid **230**, e.g., by use of a stepper motor **250**. A valve **270** is then closed, and the syringe **240** dispenses the working fluid **230** via micro-tubing **220** to a micro-pipette **260**. Since the porous polymer Bragg grating absorbs approximately 10-100 nL of fluid at a time, the micro-tubing **220** delivering the working fluid **230** to the micro-pipette fluid dispensers **260** may be similarly sized to catheter tubes. The micro-pipette fluid dispensers **260** hold approximately 1 μL of fluid and are capable of accurately dispensing approximately 10-100 nL of working fluid. The micro-pipette fluid dispensers **260** dispense the solution to the detector modules **280** of the DMA for identification of a target agent. The number of micro-wells **200** and fluid dispensers **260** in the array **210** range

in size from at least one to hundreds, depending on the number of agents that need to be monitored.

[0062] Referring to **Fig. 3**, a multi-channel DMA **300** includes at least one to hundreds of individual detector modules or chips (hereafter "modules") **305**, each designed to respond in a highly specific manner to one particular target agent. If one module **305** detects the presence of the particular target agent, then the specific module **305** reporting the decision automatically determines the identification of that target agent. A specific conjugate molecule or detector molecule (e.g., antigen or antibody) (hereafter "detector molecule") bound to a grating, that in turn binds the target agent within the working fluid, determines agent specificity. With the exception of specific detector molecules per detector module, all other features of each module **305** are identical.

[0063] The detector module **305** identifies agents in the working fluid **50**. Micro-pipette dispensers **310** at a sample arm inlet reservoir **311** and control arm inlet reservoir **312** on the module **305** introduce the working fluid **50** containing potentially hazardous agents, as well as other inert material, to the module **305**. Each reservoir **311**, **312** typically holds 100-1000 nL of solution. Micro-fluidic channels **315** having exemplary cross sectional areas of approximately $10\times10\ \mu m^2$ to $100\times100\ \mu m^2$, direct the solution, i.e., working fluid and inert materials, in the sample and control arms to porous polymer Bragg gratings **320**, **321**. Pressure gradients, or alternatively, electrokinetics, may induce fluid flow within the micro-fluidic channels **315**. In the case of electrokinetic inducement, additional electrodes in the module move the solution along the channel by electrophoresis and/or electroosmosis. The porous Bragg gratings **320, 321** allow the solution to flow through. An outlet micro-pipette **325** collects the material at the outlet reservoir **326**. Proper filtering in the collection subsystem **20** and dispenser subsystem **60** of **Fig. 1** ensures that particles are small enough to pass through the pores of the gratings **320, 321**. However, sample grating **320** acts chemically as a "selectively sticky" filter through detector molecules that are bound in the pores and/or on the surface of the sample grating **320**. Inert materials flow through the filters of subsystems **20** and **60** and the sample grating **320**, but target agents are selectively bound to a corresponding detector molecule and become a permanent part of the grating. Optically, the porous polymer Bragg gratings **320**, **321**, **322** act as narrow band

spectral filters with a reflection or transmission notch that is highly sensitive to refractive index changes. The selective binding modifies the refractive index of the grating. The control grating **321** has no detector molecules and hence does not selectively trap any target agents. All material that is not trapped is swept through the filters to the outlet reservoir **326** and then through the outlet micro-pipette **325**. When a particular agent is present in the solution, the ID subsystem **70** is sensitive to the refractive index changes in the gratings **320**, **321**. If the refractive index changes, a transduction mechanism then provides a signal to alert the system **70** of the presence of an agent.

[0064] In addition to the Bragg gratings **320**, **321**, **322** described above, each module **305** consists of an optical channel waveguide **335** comprised of three arms: (1) a sample arm **330**, (2) a control arm **331**, and (3) a reference arm **332**. All three arms contain identically fabricated porous polymer Bragg gratings **320**, **321**, **322**. The porous polymer Bragg gratings **320**, **321**, **322** have been integrated onto the module along with a light source **340**, waveguides **335**, waveguide splitters **345**, **346**, micro-fluidic channels **315**, photodetectors (i.e., photodiodes) **350**, **351**, **352** and processing electronics **360**. The response from each arm **330**, **331**, **332** is continuously and simultaneously monitored and processed electronically to factor out environmental disturbances, including inert material in the sample, to achieve a high sensitivity and a low false alarm rate.

[0065] Light is launched into the waveguide from the light source **340**, which may be a broadband light emitting diode ("LED") or preferably a single-frequency laser diode ("LD"). At the first Y-splitter **345**, a portion of the light is directed to the reference arm **332** containing a reference grating, and subsequently, a photodetector **352**. The reference grating is hermetically sealed; it never is exposed to the working fluid **50**. The reference grating may or may not contain a pure solution in its pores. The optical properties (e.g., index of refraction) of the reference grating change only due to thermal changes in the system. The light detected by the photodetector **352** in this arm **332** changes only due to thermally induced changes in the reference grating, energy fluctuations, or drift of the light source. The remaining energy at the first Y-splitter **345** is directed to a second Y-splitter **346** where the energy is split into two equal parts and directed to the sample **330** and control **331** arms of the detector module **305**.

[0066] The sample **330** and control **331** arms contain the activated sample grating **320** and the unactivated control grating **321**, respectively. The location of the porous polymer Bragg gratings **320**, **321**, **322** may be in the channel waveguide or in the waveguide cladding that form the sample **330**, control **331**, and reference **332** arms. Each grating is constructed to reflect light at the same wavelength over a very narrow spectral band. Foreign material passing through the filters produces modifications to the refractive index. The modifications shift the spectral location of the reflection notch and produce a change in the transmitted light detected by the photodetectors **350**, **351**. Energy detected at the photodetectors **350**, **351** will also change due to thermal drift of the reflection notch or light source fluctuations. The fluctuations are removed by taking the difference of the sample grating **320** and control grating **321** signals. Alternatively, the processing electronics **360** can subtract the reference grating arm **332** signal from both the sample grating **330** and control grating **331** arm signals. Since all three gratings **320**, **321**, **322** and their respective photodetectors **350**, **351**, **352** are located on the same module **305**, the gratings **320**, **321**, **322** experience the same fluctuations due to thermal drift, light source fluctuations, and other disturbances. This process thus removes spurious signals due to the detector environment. The reference grating arm **332** can also maintain the wavelength of the light source tuned to the Bragg grating **322**. As the Bragg grating notch drifts due to thermal drift, the signal from the reference grating arm **332** passes through a feedback loop to the light source **340** to tune the wavelength of the source to the notch wavelength of the Bragg grating **322**. The remaining signals are the result of foreign material, i.e., agents, present in the working fluid. Inert material passes through the gratings and produces transient changes in the refractive index. As a result, transient signal responses are produced at the photodetectors **350**, **351**. The signals from the sample grating **330** and control grating **331** arms are integrated over an appropriate time interval (e.g., by sample and hold circuits) and then subtracted (e.g., by a differential amplifier). Since both arms, **330** and **331**, identify, on the average, the same amount of inert material, these signals will cancel, producing a null signal. However, if target agents, i.e., molecules, are present, the target agents stick to the sample grating **320** and permanently change its refractive index. Moreover, the refractive index change increases with each captured target agent. Since the reflection notch is spectrally narrow, a relatively small change in

refractive index produces a large change in filter transmittance. The subsequent change in the transmitted light over the integration interval upsets the balance in the two arms **330** and **331**. The difference signal is passed through a differential amplifier in the electronic processor **360**. The presence of a non-zero signal heralds the presence of the target agent. Once a target agent detection is accomplished, that specific detector module **305** is replaced before the system is used in another monitoring scenario.

[0067] In addition to the component parts of the detection module **305** shown in **Fig. 3**, **Figs. 4** and **5** provide additional description of component parts according to an embodiment of the present invention. Referring to **Fig. 4**, detection module **405** includes a light source **440**, a channel waveguide **435**, gratings **420**, **421**, **422**, a micro-fluidic circuit **415**, photodetectors **450**, **451**, **452**, and processing electronics. The processing electronics are not shown in **Figs. 4** and **5**. The module **405** resides on a silicon wafer **470**. Light source **440**, photodetectors **450**, **451**, **452**, and large scale integration processing circuitry are either mounted directly on the silicon wafer **470** or integrated monolithically with the silicon wafer **470**, at electrodes **475**, **476**, **477**, **478**. The silicon wafer **470** is coated with a silicon dioxide (SiO_2) layer **480**. A silicon oxynitride (SiON) channel waveguide **435** along with sample **445**, reference **446**, and control **447** arms, resides in this layer. Micro-fluidic circuit **415**, as well as the fluid input **411**, **412** and output **426** ports, are etched in the SiO_2 layer **480**. The SiO_2/SiON channel waveguide **435** and micro-fluidic circuit **415** are covered with a glass slab **490**.

[0068] The Bragg gratings **420**, **421**, **422** are situated in the channel waveguide **435**. To form the Bragg gratings **420**, **421**, and **422**, a rectangular cavity is etched in the channel waveguide **435** at the position selected for each of the gratings. This cavity is then filled with a pre-polymer syrup described herein, and a porous polymer grating is formed by the procedures discussed further with respect to at least **Figs. 14a-14c**. Micro-fluidic circuit **415** is etched in the SiO_2 layer **480** as shown, and the micro-fluidic channels are directed in a substantially perpendicular direction to the long dimension side of the gratings **420**, **421**. In this manner, the working fluid of the device will flow through the porous gratings **420**, **421** at an angle substantially perpendicular to the channel waveguide **435** axis. In one embodiment, the surface of layer **480** is exposed to air. However, in an alternate embodiment, the SiO_2/SiON waveguide circuit **435** and

micro-fluidic circuit **415** are covered with a glass sheet **490** to seal the micro-fluidic circuit **415** and the porous polymer Bragg gratings **420**, **421**, **422** from the environment, e.g., air. Small pilot holes **491**, **492**, **493** are etched in the glass **490** to match up with the inlet/outlet ports **411**, **412**, **426** of the micro-fluidic circuit **415** on the module **405**. An electrical feed-through **495** is also provided for reference photodetector **452**.

[0069] Referring to an alternative embodiment in **Fig. 5**, detection module **505** resides on a silicon wafer **570**. A SiO_2 layer **580** is coated with a polymer layer **590**, such that the polymer has an index of refraction smaller than the SiON channel waveguide **535**. Porous polymer Bragg gratings **520**, **521**, **522** are situated in the polymer cladding layer **590**. Micro-fluidic circuit **515** is etched in the polymer cladding layer **590**. The fluid input **511**, **512** and output **526** ports are likewise etched in the polymer layers **590**. The polymer cladding **590** includes an electrical feed-through **595** for the reference photodetector **552**. A laser diode **540** and photodetectors **550**, **551** contact electrodes **575**, **576**, **577**.

[0070] Alternatively, the Bragg gratings **520**, **521**, **522** are situated in the cladding directly above the channel waveguide **535**. The SiO_2 layer **580** containing the SiON channel waveguide **535** is coated with a polymer cladding **590**. A rectangular cavity is etched in the polymer cladding **590** at the positions selected for the gratings. This cavity is then filled with a pre-polymer syrup described herein, and a porous polymer grating is formed by the procedures discussed further with respect to at least **Figs. 14a-14c**. Micro-fluidic circuit **515** and inlet/outlet ports **511**, **512**, **526** are etched in the polymer cladding **590** as shown, and the micro-fluidic channels are directed in a substantially perpendicular direction to the long dimension side of the gratings **520**, **521**. In this manner, the working fluid of the device will flow through the porous gratings **520**, **521** at an angle substantially perpendicular to the channel waveguide **545** axis. The polymer cladding **590** is exposed. Optionally, the polymer cladding **590** is covered with a glass sheet to seal the micro-fluidic channels **515** and the porous polymer Bragg gratings **520**, **521**, **522** from the outside environment. Small pilot holes are etched in the glass to match up with the inlet/outlet ports **511**, **512**, **526** of the micro-fluidic circuit in the polymer cladding **590**.

[0071] With respect to **Figs. 4** and **5**, the light sources **440** and **540** are preferably a laser diode ("LD"). In particular, tertiary semiconductor lasers, such as $Al_xGa_{1-x}As$, and quaternary

semiconductor lasers, such as $In_{1-x}Ga_xAs_{1-y}P_y$, are useful, where the ratios x and y can be varied to adjust the laser wavelength. AlGaAs lasers generally provide wavelengths between 750 nm and 870 nm. $In_{1-x}Ga_xAs_{1-y}P_y$ lasers generally provide wavelengths between 1.1 μm and 1.6 μm. Visible wavelength lasers, such as GaInP (670 nm) and AlInP (584 nm), may also be useful. The LD is bonded upside-down to an electrode on the silicon surface and butt-coupled to the SiON channel waveguide.

[0072] Further, waveguides **435**, **445**, **446**, **447**, **535**, **545**, **546**, and **547** may be formed of SiON as a core material. Depending on the nitrogen-to-oxygen ratio, the refractive index of SiON can be varied between 1.46 and 2.3. Thus, the SiON refractive index can be tuned to be greater than that of SiO_2 to form a waveguide. And, the refractive index can be matched to that of the polymer (approximately 1.52) used in the inline Bragg gratings, or tuned to be slightly larger than the polymer that is used as the cladding layer. Thus, it is possible to tune the index so that the porous polymer Bragg gratings can be situated directly in the channel waveguide, or in the waveguide cladding directly above the channel waveguide as described herein.

[0073] In further reference to **Figs. 4** and **5**, the optical outputs of the reference **447**, **547**, sample **445**, **545**, and control **446**, **546** waveguide channels are coupled to photodetectors **450**, **451**, **452**, **550**, **551**, **552**. The photodetectors **450**, **451**, **452**, **550**, **551**, **552** are mounted upside-down and bonded to electrode pads **475**, **476**, **477**, **575**, **576**, **577** on the silicon wafer **470**, **570**. For visible wavelengths from approximately 400 nm up to approximately 900 nm in the near infrared, silicon photodiodes may be used. For wavelengths in the range of about 900 nm to 1600 nm, AlGaAsP photodiodes may be used.

[0074] Referring to **Figs. 6a** and **6b**, an embodiment illustrates the type of electronic processing applied to the photodetector outputs for the sample and control channels of **Figs. 3**, **4**, and **5**. Referring to an embodiment in **Fig. 6a**, only inert material flows through both the sample and control porous polymer Bragg gratings. Referring to an embodiment in **Fig. 6b**, target agents, along with the inert material, pass through the gratings. In each of **Figs. 6a** and **6b**, the electronic processing system consists of four main parts: (1) sample and hold circuits **600**, **601**;

(2) differential amplifier **610**; (3) analog-to-digital (A/D) converter **605**; and (4) additional digital processing electronics **615**.

[0075] The sample and hold circuits **600**, **601** sample voltages from the photodetectors over a specified time interval. The time interval is selected by a negative pulse of a predetermined time interval applied to the gate of a p-channel MOSFET **670**, which closes a switch and allows data in the form of a stream of voltage pulses from photodiodes to pass through the switch and be stored on a capacitor **620**, **625**. These pulses, as illustrated in the embodiment in **Fig. 6a**, represent transient responses from the photodiodes due to inert material flowing through the gratings and producing fluctuations in the average refractive index. This leads to the transmission of transient light pulses through the grating that are detected by the photodiodes. The sample and hold circuits receive input from a control data stream **630**, **631** and corresponding gate pulse **635**, **636**, as well as sample data stream **640**, **641** and corresponding gate pulse **645**, **646**. The input is received through amplifiers **675**. The output of the sample and hold circuits **600**, **601**, at the end of the gate pulse, are dc voltages, V_C **650**, **651**, for the control channel, and V_S **655**, **656**, for the sample channel. V_C and V_S represent the sum of voltage spikes from their respective channel. These voltages are input to a differential dc amplifier **610** that employs one operational amplifier **660**. The conventional offset-voltage balancing circuitry standard for differential amplifiers is not shown. The high gain of the operational amplifier **660** results in an output that amplifies the difference between the two input voltages by a factor approximately equal to the ratio of R_2 **662** to R_1 **661** (e.g., if R_1=1 kΩ and R_2=100 kΩ, R_2/R_1=100). On average, the same amount of inert material is expected to flow through both control and sample gratings. Output V_{out} **665**, **666**, is equal to $(R_2/R_1)(V_S-V_C)$. Therefore, output V_{out} **665** of the differential amplifier **610** should be approximately zero. The longer the sampling interval (i.e., the longer the gate pulse), the closer this output approximates zero when only inert material flows through the porous gratings. Output V_{out} **666** does not equal zero. A standard A/D circuit **605** digitizes the output voltage. This data is stored and/or processed further. For example, averages are computed over several sampling intervals to improve the sample statistics. Other more complex data processing can also be accomplished. In the embodiment shown in **Fig. 6b**, target agents stick to the sample Bragg grating in sample data stream **641** resulting in a

non-zero persistent voltage baseline on which transients, due to inert material, ride as voltage fluctuations. The output of the differential amplifier is non-zero, thus signaling a target-molecule detection event.

[0076] The above scheme also discriminates real signals from photodiode voltage fluctuations and drift that may occur due to light source power fluctuations, thermal drift, and other environmental disturbances. Since the two detector arms reside on the same module, they are subject to the same external disturbances. Voltages produced due to these effects are common to both channels and subtracted out by the differential amplifier.

[0077] Voltage drift due to external influences may also be factored out by using the output of the reference arm of the module in a set of circuits similar to those of **Figs. 6a** and **6b**, comparing the reference output with the sample and control outputs separately. Thermal drift may cause the Bragg grating notch to wander off the laser wavelength due to thermal changes in the refractive index. To optimize operation of the Bragg gratings, the reference arm photodiode may be used in a feedback loop to tune the laser wavelength to match any thermal drift of the Bragg gratings and lock the laser wavelength to the Bragg condition. Diode lasers are available whose output wavelengths can be tuned electronically over a few nanometers.

[0078] To discriminate fluctuations and drift of the Bragg grating from those of the light source, in an alternative embodiment, the reference arm could be replaced with a dual reference arm using an additional Y-splitter. For example, referring to **Fig. 7**, a sealed porous polymer Bragg grating **710**, on an SiO_2 layer **705** substantially coating a Si substrate **700**, terminating at a photodiode **720**, and the second reference arm **716** would have no grating and terminate at a separate photodiode **721**. The outputs of the two reference arms **715**, **716** are compared to discern thermal drift of the Bragg grating **710** from fluctuations or drift of the output power from the laser diode **725**. Sample and control arms **730**, **740** are directed to gratings **735**, **745** proximate micro-fluidic channels **750**. All photodiodes **720**, **721**, **722**, **723** lead to an electronic processor **760** with an LD stabilization feedback loop **770** to the laser diode **725**.

[0079] Referring to **Fig. 25**, in an embodiment of the present invention, a porous polymer Bragg grating **2500** is situated in a channel waveguide **2510**. Light is confined to the waveguide

2510 by total internal reflection at the substrate **2550** and cladding **2540**. Light at the Bragg wavelength propagating along the waveguide **2510** is reflected from the grating **2500** and propagates back along the waveguide **2510** in the opposite direction. Therefore, transmitted light at the Bragg wavelength is attenuated.

[0080] In an alternative embodiment of the present invention, referring to **Fig. 9**, a porous polymer medium serves as cladding **940** for a corrugated waveguide **910**. In order to form cladding **940**, the pre-polymer syrup is cured using a single incoherent beam of light such that no grating is recorded. The phase-separated liquid crystal is then removed to yield the porous polymer cladding. The corrugated waveguide **910** acts like a Bragg grating. Incident light is shown as **960** and reflected light is shown as **970** within **Fig. 9**. Light evanescently coupled to the porous cladding **940** will sense any change in the refractive index of the cladding **940**. This would produce a spectral shift of the reflection notch of the filter. Changes in the refractive index of the grating change the efficiency of the evanescently coupled light.

[0081] Changes in the refractive index of the grating affect the efficiency of the coupling at a specific wavelength. Referring to **Fig. 10**, an alternative embodiment places a porous polymer Bragg grating **1000** in the cladding **1040**. Guided light **1060** through waveguide **1010** experiences spectrally selective loss to radiation modes **1070** by evanescently coupling to the Bragg grating **1000** in the cladding **1040**. Referring to **Fig. 11**, two channel waveguides **1110**, **1115** are coupled evanescently to form a waveguide coupler, directing out-coupled light **1170** and input light **1160**. A holographic grating **1100** in the cladding region **1140** serves to spectrally assist this coupling for light at the Bragg wavelength. Out-coupled light **1170** is directed into the opposite direction in the output waveguide **1110**. This configuration prevents reflected light from impinging back on the light source. Referring to **Fig. 12**, a long period, i.e., where Λ is substantially greater than λ, porous polymer grating **1200** is placed in a waveguide **1210** substantially on a substrate **1250**. Such a grating **1200** serves to resonantly couple light **1260** at a specific wavelength to radiation modes **1270**. Referring to **Fig. 13**, an asymmetric waveguide **1310** has a Bragg reflection grating **1300** serving as the substrate. Light **1360** resonantly coupled to the Bragg condition of the grating **1300** follows a zigzag path down the slab waveguide **1310**.

[0082] In an alternative embodiment, a single Bragg grating in a waveguide channel can be used as a stand-alone detector element. Referring to top and side views, **Fig. 8a** and **Fig. 8b**, a porous polymer Bragg grating **800** is situated in a channel waveguide **810**, as a stand-alone element, optically coupled to input optical fibers **820** and output optical fibers **830**. Waveguide **810** and grating **800** are proximate to substrate **850**. An LED or LD source **860** launches a wave into the fiber **820**, which is coupled into the Bragg grating **800** and subsequently out-coupled to a miniature spectrometer (not shown), such as the S2000 manufactured by Ocean Optics Inc. The spectrometer monitors any real-time changes to the diffraction efficiency of the grating **800**.

[0083] As referenced previously herein, certain embodiments of the present invention utilize polymer-dispersed liquid crystal ("PDLC") or holographic PDLC ("HPDLC") related technology in the formation of the Bragg gratings and waveguide components. Descriptions of PDLC materials and related technology can be found in U.S. Patent No. 5,942,157, U.S. Patent Application Serial No. 09/363,169 filed on July 29, 1999 for Electrically Switchable Polymer Dispersed Liquid Crystal Materials Including Switchable Optical Couplers and Reconfigurable Optical Interconnects, U.S. Patent Application Serial No. 10/235,622 filed on September 6, 2002 for Electrically Switchable Polymer Dispersed Liquid Crystal Materials Including Switchable Optical Couplers and Reconfigurable Optical Interconnects, U.S. Application Serial No. 10/303,927 filed on November 26, 2002 for Tailoring Material Composition for Optimization of Application-Specific Switchable Holograms, and U.S. Patent Application Serial No. 60/432,643 filed on December 12, 2002 for Switchable Holographic Polymer Dispersed Liquid Crystal Reflection Gratings Based on Thiol-ene Photopolymerization, each of which is incorporated by reference herein in its entirety. In a preferred embodiment of the present invention, the Bragg gratings comprise static holograms formed through holographic polymerization of a PDLC material using coherent light beams. As is described above with reference to **Figs. 14a-14c**, after holographic polymerization, the liquid crystal is removed from the film. Extraction of the liquid crystal leaves pores within the remaining polymer matrix approximately 100 nm in diameter. The pores within the polymer matrix contain binding sites, such as COOH or NH_2, for a detector molecule. In a particular example, the polymer may be thiol-ene, thiol-acrylate, or one of various multifunction acrylates described in U.S. Patent No. 5,942,157. Depending on the polymer

composition, the polymer can be cured via visible or ultraviolet ("UV") laser radiation. The evacuated polymer matrix is then chemically treated to activate the binding site for the detection molecule. The activation procedure depends on the functional group of the binding site. For example, if the functional group is COOH, then amine coupling, e.g., EDC (N-ethyl-N'-(3-dimethyl aminopropyl)-carbodiimide hydrochloride) and NHS (N-hydroxysuccinimide), is an accepted procedure commonly referred to as EDC/NHS coupling procedure. Alternatively, for the activation of NH_2, an accepted procedure is cross-linking to free amino groups via polymerized gluteraldehyde. Any method that activates the binding site to bind to a detector molecule is sufficient. Next, a detector molecule is bound to the activated binding site. This detector molecule then can sense a target agent of interest. The detector molecule can be an enzyme, a protein, an antibody, or an antigen. The detector molecule is specifically selected to bind to the target agent. After activation and detector molecule attachment, the binding sites that are unattached to any detector molecule are blocked using conventional means. The blocking is usually achieved with a large protein, such as casein or BSA. When the target agent binds to the detector molecule, it causes a change in the refractive index of the polymer, thus causing a shift in the wavelength of the holographic notch.

[0084] In a further embodiment of the present invention, described herein is the structure and formation of the Bragg gratings. Referring to Fig. **14a**, a pre-polymer syrup consisting of a mixture **1405** of polymerizable monomer, photoinitiator, co-initiator, liquid crystal, binding site monomer, and in alternative embodiments, a cross-linking monomer and a long chain aliphatic acid are spread in a thin layer, typically about 10 μm, in slab waveguide format on a substrate **1410**. In this embodiment of the present invention, two coherent beams of light **1415** are incident on the mixture **1405**, forming a pattern consisting of PDLC **1420a** and polymer **1420b** channels within the mixture **1405**, as shown in **Figs. 14b** and **14c**. In this embodiment, the polymerized material **1420** is referred to as HPDLC material, since the overlapping coherent beams **1415** form an interference pattern within the photopolymerized material **1420**. During photopolymerization, portions of monomer from mixture **1405** are consumed forcing the remaining monomer to diffuse and replace the consumed portions of monomer, forming polymer channels **1420b**. This diffusion displaces liquid crystal in mixture **1405** since it does not

participate in the photochemical reaction, and the liquid crystal diffuses to channels **1420a**. As the local liquid crystal concentration increases over time, the miscibility gap for the liquid crystal/polymer solution is eventually breached, and the liquid crystal separates out as a distinct phase in channels **1420a**. A more detailed description of this process is found in U.S. Patent No. 5,942,157.

[0085] Referring to **Fig. 14b**, the liquid crystal phase takes the form of interconnected nanoscale droplets of liquid crystal **1425** within a polymer matrix **1430**. The nanoscale domains of liquid crystal **1425** are of controllable density and size. The liquid crystal **1425** is then extracted by: (1) soaking the hologram with a solvent followed by drying; (2) vacuum evacuating the pores; or (3) a combination of (1) and (2).

[0086] Referring to **Fig. 14c**, as a result of the liquid crystal extraction from the polymer matrix **1430**, there remains an open structure of interconnected voids or pores **1435** (hereafter "pores"), where the pores **1435** are periodically distributed in the photopolymerized material **1420**. The resulting hologram with pores **1435** is then treated chemically to activate the binding sites **1450** then attach detector molecules **1440** (i.e., antibodies) to the activated binding sites **1450**. As described further herein, antigens **1445** may attach to detector molecules **1440**. The Bragg grating formed by this process may then be used in the detector modules described with reference to, for example, **Figs. 5 – 7**, in order to detect target agents within the working fluid. The polymer matrix **1430** has a refractive index of about 1.52, while that of the aqueous solution is approximately 1.33. The periodic index mismatch creates an index modulation or an optical grating. The grating exhibits Bragg diffraction for light at a specific wavelength propagating at a specific angle of incidence. For light propagating in the plane of the film, the hologram is a Bragg reflection grating that retro-reflects light at the Bragg wavelength. The Bragg wavelength is determined by the average refractive index and the periodic spacing of the porous regions. The strength of the reflection, i.e., the diffraction efficiency, is determined by the magnitude of the index modulation and the physical length of the filter, i.e., region of index modulation. Generally, the diffraction efficiency will become larger with increased filter thickness, and the spectral width of the reflection notch will decrease with increased filter thickness. The index modulation is determined by the difference in refractive indices of polymer matrix and aqueous

solution, which in this exemplary embodiment is approximately 0.19, and the density of pores. A low index modulation can thus be achieved by a low density of pores. In a preferred embodiment, a small density of pores, i.e., low index modulation, and a thick filter is achieved in a waveguide configuration, resulting in a spectrally narrow filter with large diffraction efficiency. Nonetheless, the specific pore density must be consistent with a sufficient flow rate of working fluid through the hologram. Specific grating compositions are described further below.

[0087] The selected Bragg wavelength is determined by such factors as the chosen laser wavelength and the spectral region of sensitivity desired for detecting a refractive index shift based on the polymer and detector molecule reaction selected. This optical region may be anywhere across the visible or near infrared spectrum. The grating period Λ is selected by forming a hologram with a recording wavelength λ_r and an angle of incidence θ_r of the incident beams, with $\Lambda=\lambda_r/2\sin\theta_r$. Thus, either λ_r or θ_r, or both, can be varied to form the desired grating period. The Bragg wavelength λ_B for light propagating substantially along the waveguide axis is approximately $2n\Lambda$, where n is the average refractive index of the medium at λ_B. The index n changes as target agents are bound to the polymer matrix. The Bragg wavelength λ_B is given by:

$$\lambda_B = 2n\,(\lambda_r / 2\sin\theta_r)$$

[0088] The bandwidth $\delta\lambda$ of the spectral diffraction efficiency for a Bragg grating is given by:

$$\delta\lambda = \frac{\lambda_B^2}{\pi n}\sqrt{\kappa^2 + (\pi/L)^2}$$

where κ is the coupling constant of the grating and L is the grating thickness. The coupling constant is further given by $\kappa=\pi n_1/\lambda_B$, where n_1 is the amplitude of the index modulation of the grating. For sufficiently thin gratings, the bandwidth is inversely proportional to the thickness. Thus, a thicker grating leads to a sharper reflection notch. The thicker grating also increases the diffraction efficiency. For sufficiently thick gratings, the bandwidth is directly proportional to κ. Thus, a small coupling constant κ (i.e., a small index modulation) also leads to a narrow spectral notch. Generally, a thick filter with a small index modulation yields a grating with high peak diffraction efficiency and a narrow spectral notch.

[0089] The index modulation of the grating is produced by the periodic variation of nanoscopic pores throughout the volume of the polymer. Typically, the density of pores has the form of a rectangular wave, with a volume fraction of pores f_c in a channel of width $\alpha\Lambda$, ($0<\alpha<1$) and no pores in adjacent channels of width $(1-\alpha)\Lambda$. The index modulation is related to the first Fourier component of the Fourier expansion of this rectangular wave, and is given by:

$$n_1 = \frac{2f_c}{\pi}\sin(\alpha\pi)(n_p - n_s)$$

where n_p and n_s are the refractive indices of the polymer and solution filling the pores, respectively. The parameters f_c and α are determined by the phase separation of liquid crystal during the recording of the holographic grating. These are controlled by processing parameters such as recording intensity and total exposure, as well as material properties including liquid crystal concentration and concentrations of other recipe constituents, such as long chain aliphatic acids. With the refractive indices relatively fixed at n_p approximately equal to 1.52 and n_s approximately equal to 1.33, the index modulation is directly controlled by the values of f_c and α.

[0090] Referring to **Fig. 15**, in an exemplary embodiment of the present invention, the spectral diffraction efficiency of a grating with f_c=0.4, α=0.3, and L=25 μm is shown. The index modulation n_1 is 0.039. Alternatively, referring to **Fig. 16**, a filter response with the same peak efficiency has a reduced bandwidth. For this grating, f_c=0.1, α=0.3, and L=100 μm. The corresponding index modulation n_1 is 0.0098. A filter at least this thick is readily achieved in a waveguide configuration. A sharper reflection notch gives a more sensitive change in transmittance with changes in refractive index. A holographic recording method for creating a porous polymer grating enables users to set processing parameters in order to easily obtain the index modulation for the sharp reflection notch.

[0091] The presence of sidelobes **1600**, such as those shown in **Fig. 16**, in certain applications leads to ambiguous signals. Sidelobes **1600** are naturally occurring features of all volume gratings with uniform index modulation. Inducing a non-uniform index modulation in the grating eliminates sidelobes, a process called apodization. Apodization is readily achieved using holographic techniques. Referring to **Fig. 17**, the plot exemplifies the spectral efficiency of a grating with Gaussian apodization. This filter has the same peak efficiency as the embodiment in

Fig. 16, but is sharper and has no sidelobes. For this grating, L=280 μm. The peak index modulation in the middle of the grating is n_1=0.0098. However, the index modulation amplitude falls off as a Gaussian function toward the front and back of the grating. Such a grating is obtained holographically by giving the recording beams a Gaussian intensity distribution.

[0092] As exemplified in **Fig. 18a**, Gaussian intensity distribution is achieved by passing the beams **1815** through at least one neutral density filter **1800**, over a pre-polymer syrup mixture **1805** on a substrate **1810**, with optical density that has an inverse Gaussian distribution. Other filters or masks can produce other types of apodizing functions, such as raised cosine, hyperbolic tangent, polynomial, or other apodizations based on mathematical distributions. The transparency of the at least one neutral density filter **1800** substantially decreases in the direction of arrows **A**. Referring to **Figs. 18b** and **18c**, since the pore density that forms in the PDLC hologram **1820** is a function of the local intensity, resulting grating **1822** has a pore distribution that follows the local intensity of the recording beams **1815**. The transparency of the PDLC hologram **1820** substantially increases in the direction of arrows **B** as shown in **Fig. 18b**. Referring to **Fig. 18c**, the resulting grating **1822** has alternate polymer slabs **1830** and porous slabs **1835**. The pore density of the porous slabs **1835** substantially decreases in the direction of arrows **C**.

[0093] Sensitivity is built into the detection in two ways. First, there is an optical differential gain. The filter reflection or transmission notch is very sharp spectrally and exhibits a large change in transmittance for a relatively small change in refractive index. Second, there is an electronic differential gain. Signals from the sample and control arms are detected and processed in an electronic differential amplifier that produces a large output for a relatively small difference between the two signals. See **Figs. 6a** and **6b** for processing electronics.

[0094] To build specificity into the sample gratings, the binding sites of the pores or polymer matrix of the holograms are chemically activated and the detector molecules are bound to the polymer matrix. Thus, if a target agent is present in the working solution, it will selectively bind to the detector molecules. The target agent becomes trapped in the pore. Since the chemical

nature of the polymer matrix changes, the average refractive index also changes. Consequently, the spectral properties of the porous polymer Bragg grating also change.

[0095] Bound target agents modify the spectral properties of the sample grating by changing the refractive index and possibly swelling the polymer. The diffraction efficiency of the sample grating is very sensitive to these changes. The spectral shift $\Delta\lambda_B$ of the grating is determined by

$$\frac{\Delta\lambda_B}{\lambda_B} = \frac{\Delta n}{n} = \frac{\Delta\Lambda}{\Lambda}$$

For example, let the Bragg wavelength be λ_B=850 nm for a grating with an average index n=1.5 and grating period Λ=283 nm. Thus, a spectral shift of $\Delta\lambda_B$=1 nm is produced by $\Delta n/n=\Delta\Lambda/\Lambda=0.0012$, i.e., Δn=0.00183, or $\Delta\Lambda$=0.33 nm. A spectral shift of 1 nm produces a very significant change in the transmittance of the filter. Referring to **Fig. 19**, three transmission notches are shown for three different refractive indices for a filter irradiated by a broadband light source, e.g., LED. The center notch **1920** for n=1.5000 is centered spectrally at λ=0.850 μm or 850 nm. Transmission notch **1910** shifts with a decrease of index, while transmission notch **1930** shifts with an increase in index. The effects are similar to the swelling of polymer, i.e., a change of grating period. An index change of $\Delta n=\pm0.0025$ shifts the notch by greater than 1 nm and changes the transmission substantially, from about 5% to about 100%, at 850 nm.

[0096] The sensitivity of the sample grating may be enhanced by interrogating the grating at a wavelength near a region of rapidly changing efficiency. The diffraction efficiency η (i.e., reflection; transmission equals 1-η) for a Bragg grating is given by:

$$\eta = \tanh^2(\kappa L)$$

where κ is the coupling coefficient and L is the filter thickness. The relative change $\Delta\eta/\eta$ in diffraction efficiency is given by:

$$\frac{\Delta\eta}{\eta} = 8.4\frac{\eta}{\kappa L}\frac{L}{\lambda_L}\Delta n$$

and

$$\frac{\Delta\eta}{\eta} = 16.8n^2\frac{\eta}{\kappa L}\frac{L}{\lambda_B^2}\Delta\Lambda$$

for changes in refractive index and grating period, respectively. Referring to **Fig. 20**, κL=2.1, L=2 mm, λ_B=850 nm, and n=1.5. The laser wavelength λ_L **2010** is approximately 850.075 nm. A readily detectable change in efficiency **2020** of $\Delta\eta/\eta$=0.05 is achieved with just Δn=6.5×10^{-6} or $\Delta\Lambda$=1.2×10^{-3} nm. Referred to as optical differential gain, a relatively small change in index or period produces a relatively large change in transmittance.

[0097] Referring to **Fig. 21a**, as another exemplary embodiment, the laser wavelength **2110** is at a null in the diffraction efficiency. A small change in refractive index then produces a rapid rise in the diffraction efficiency **2120** from a zero background. The benefit of this effect is realized when the photodetector is placed to view reflected light rather than transmitted light. Referring to **Fig. 21b**, the embodiment is exemplified as a plot of diffraction efficiency η as a function of Δn at λ = 0.8501448 μm.

[0098] The following embodiments are set forth herein to describe exemplary materials and grating configurations that were useful as detectors in experiments conducted in free space. One skilled in the art recognizes the configuration changes necessary to incorporate such examples into the waveguide embodiments contemplated by the present invention.

[0099] In a first exemplary embodiment, gratings are constructed using thiol-acrylate as the polymerizable monomer and 2-carboxyethylacrylate (2-CEA) as the binding site monomer, with 10-μm thickness achieved by sandwiching the mixture between two glass plates. These gratings have one glass substrate coated with a release agent. The release agent substrate is removed, and the gratings are evacuated in a vacuum oven over a period of approximately three days to remove the liquid crystal. The cells are scanned in, for example, a Cary 500 UV/VIS spectrophotometer from Varian. The sensor molecule Gliadin is bound to the grating to sense the anti-Gliadin target agent. Before attachment of Gliadin to the carboxy (COOH) group of the HPDLC, the carboxy group is activated using the EDC/NHS coupling procedure. After activation of the groups, the Gliadin is attached. Following attachment, another protein, casein, is used to block carboxy groups that were not attached to Gliadin. Casein does not interfere with the target agent attachment, since the anti-Gliadin antibodies are specific for Gliadin.

[0100] In a second exemplary embodiment, a batch of HPDLC gratings are made on BK7 optical flats at 25-μm thickness. The gratings are evacuated over approximately three days to remove the liquid crystal. Certain gratings from the batch are checked with an ELISA (Enzyme Linked Immuno Specific Assay) kit, and other gratings are scanned in a spectrometer for a peak shift. The ELISA checked gratings and the spectrometer scanned gratings are tested with either standard A or standard F. The pre-selected spectral absorbance is proportional to concentration, i.e., A is the lowest concentration and F is the highest. The HPDLC gratings withstand treatment with all the solutions needed for protein and antibody attachment without degrading.

[0101] Exemplary embodiments involve a grating sensing anti-Gliadin, or an alternative embodiment sensing Cortisol. Materials used in the exemplary embodiments include the monomer dipentaerythritol hydroxy penta acrylate (DPHPA), photoinitiator dye Rose Bengal (RBAX), co-initiator *N*-phenylglycine (NVG), monomer *N*-vinylpyrrollidone (NVP), long-chain aliphatic acid dodecanoic acid (DDA), binding site monomer 2-carboxyethylacrylate (2-CEA), and liquid crystals E7 and TL213 (both available from Merck).

[0102] Gliadin, an antigen derived from wheat, is utilized as the sensor molecule to detect the presence of anti-Gliadin. The recipe includes 47.9% DPHPA, 0.6% RBAX, 1.5% NPG, 10.0% NVP, 38.0% E7, and 2.0% 2-CEA. The above formula, less the 2-CEA, is a conventional formulation for recording HPDLC gratings, and is given the designation CS573. A holographic recording in such a mixture results in a periodic distribution of interconnected liquid crystal droplets. The addition of 2-CEA provides COOH groups attached to the polymer matrix, with some COOH groups residing at the polymer/liquid crystal droplet interfaces. The pre-polymer mixture also includes 15 μm glass rods that act as spacers for the holographic cell which comprises two 1″-diameter, 1/8″-thick glass windows. A sonicator homogenizes the mixture prior to sandwiching the pre-polymer mixture within the holographic cell, between the glass windows. At least one of the glass windows is coated with a release agent to facilitate removal of one substrate after holographic recording. Alternatively, reflection holograms are recorded (in cells designated CS573-x, where x=1...9) using two 532-nm beams derived from the same frequency-doubled Nd:YVO_4 laser at an optical power of approximately 15 mW/cm^2 for 30 seconds. A 1-hour white-light post-cure procedure bleaches the remaining RBAX dye.

[0100] In a second exemplary embodiment, a batch of HPDLC gratings are made on BK7 optical flats at 25-μm thickness. The gratings are evacuated over approximately three days to remove the liquid crystal. Certain gratings from the batch are checked with an ELISA (Enzyme Linked Immuno Specific Assay) kit, and other gratings are scanned in a spectrometer for a peak shift. The ELISA checked gratings and the spectrometer scanned gratings are tested with either standard A or standard F. The pre-selected spectral absorbance is proportional to concentration, i.e., A is the lowest concentration and F is the highest. The HPDLC gratings withstand treatment with all the solutions needed for protein and antibody attachment without degrading.

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[0103] Following post-cure, the release-agent-coated flat is separated from the photopolymerized material, and the transmittance spectrum of each photopolymerized material is measured using, for example, a Cary500 UV/VIS/NIR spectrometer. The nominal peak of the reflection notch (minimum of the transmittance curve) is around 535 nm. The holograms are placed in a vacuum oven (approximately 28 mm Hg) for a period of about 48 hours to extract the liquid crystal. After removing the holograms from the oven, the samples are rinsed in methanol and replaced in the oven for 3 hours. Each cell is then measured again in the Cary500. All samples exhibit a blue shift of the diffraction peak, indicating that the liquid crystal is removed, and the refractive index of the composite medium is decreased.

[0104] Following these measurements, six cells (x=2, 3, 5, 6, 8, 9) are selected for further tests and one additional cell (x=1) is reserved for a control experiment. The set of six cells are divided into two subsets of three (subset A: x=3, 5, 6 and subset B: x=2, 8, 9). Table 1 below describes the procedures applied to the cells. The binding of present antibodies, formation of the sandwich complexes, and enzymatic color reaction take place during three different reaction phases.

[0105] In Phase I, solution samples containing different concentrations of target molecules are pipetted onto the Bragg gratings. Any present target agents bind to the inner surface of the Bragg grating. After a 30-minute incubation the grating is washed with wash buffer for removing non-reactive components. Phase I includes incubation with Gliadin (I w/G) or no incubation with Gliadin (I w/o G).

TABLE 1
Processing of Bragg Gratings for Gliadin—Anti-Gliadin Tests[a]

Subset	Grating	Phase I w/ G	Phase I w/o G	IgA Incubation[b]	Phase II	Phase III
A	3	x		6 U/mL		
A	5	x		6 U/mL	x	x
A	6		X	6 U/mL	x	x
B	2	x		31 U/mL		
B	8	x		31 U/mL	x	x
B	9		X	31 U/mL	x	x
Control	1			NA	x	x

[a]'x' denotes that part of the procedure that was used.
[b]Units/milliliter (U/mL) of IgA are the concentration units used by the supplier of the test kit.

[0106] After the Phase I treatment, sample Bragg gratings in subsets A and B are air-dried overnight. All sample Bragg gratings exhibit a red shift of the diffraction notch, with the non-Gliadin samples having about twice the shift of that of the Gliadin samples, due to the increased refraction index. In all cases, material, e.g., Gliadin and/or casein, is added to the vacant pores of the sample, attaching to the activated COOH sites, and thereby increasing the refractive index. The molecular weight of Gliadin is approximately 50,000 Daltons. Casein, a protein found in milk, exists most often as a micelle, with an average molecular weight of approximately 375,000 Daltons. Thus, sample Bragg gratings containing only casein have more mass and thus a higher refractive index than those sample Bragg gratings containing a mixture of casein and Gliadin. A higher refractive index implies a larger shift of the diffraction notch.

[0107] Following anti-Gliadin (IgA) incubation, sample Bragg gratings 3 and 2 are air-dried overnight. Both sample Bragg gratings exhibit an additional shift, indicating the binding of the anti-Gliadin to the Gliadin. All four spectrometer transmission scans in the above sequence for sample Bragg grating 3, as exemplified in **Fig. 22**, illustrate an initial scan **2200** after recording with liquid crystal droplets in the Bragg grating, a blue shifted scan **2210** after the pores are evacuated (liquid crystal removed), a subsequent red shift **2220** when Gliadin and casein are bound to the activated COOH sites, and a final red shift **2230** when the antibody binds to the Gliadin, thereby increasing the refractive index, i.e., adding mass to the pores. The final red shift **2230** is about 1%. Sample Bragg grating 2 exhibits a final red shift of approximately 6%, a larger shift due to a higher concentration of antibodies.

[0108] Sample Bragg gratings 5, 6, 8, 9 and control 1 are subjected to Phases II and III of the procedure. In Phase II the sample Bragg gratings are incubated in an anti-human-IgA horseradish peroxidase conjugate solution, which recognizes IgA class antibodies bound to the immobilized antigens. A wash buffer then washes away any excess enzyme conjugate not specifically bound to the antibodies.

[0109] In Phase III, a chromogenic substrate solution containing TMB (3,3′,5,5′-Tetramethylbenzidine) is dispensed onto the gratings. During incubation, the color of the solutions changes from a clear solution to blue. The addition of 1 M hydrochloric acid stops

color development to stabilize the sample for spectrometer measurements. The solution changes color to yellow. The amount of color is proportional to the concentration of IgA antibodies present in the original sample. A higher concentration of IgA produces a larger absorbance at 450 nm. The color changes of sample Bragg gratings 5, 6, 8, 9 and control 1 are quantified by measuring the absorbance of the samples at 450 nm. In all cases, sample Bragg gratings 5 and 8, both treated with Gliadin, exhibit larger absorbance than cells not treated with Gliadin (6 and 9), with the larger difference being between sample Bragg gratings 8 and 9 that are exposed to the higher concentration of IgA. The control sample Bragg grating 1 exhibits an absorbance similar to sample Bragg grating 6, which follows since neither sample Bragg grating was treated with Gliadin. Finally, a solution of TMB and HCl is formed without any exposure to the sample Bragg gratings. This solution exhibits no absorbance at 450 nm.

[0110] In another exemplary embodiment, cortisol is the antigen and the sample Bragg grating is activated with anti-cortisol to form a detector sensitive to the presence of cortisol. Cortisol is a hormone present in the body and released in higher quantities during stressed or agitated states. Designated CS576, the recipe included 51.9% DPHPA, 0.6% RBAX, 1.5% NPG, 10.0% NVP, 4.0% DDA, 30.0% TL213, and 2.0% 2-CEA. The mixture also includes 8-μm glass rods as spacers for the holographic cell, and a sonicator homogenizes the mixture. The resulting syrup is then sandwiched between two 1″-diameter, 1/8″-thick glass windows. At least one of the glass windows is coated with a release agent to facilitate removing one of the substrates. Reflection holograms are prepared using 532-nm beams. A 1-hour white-light post-cure bleaches remaining RBAX dye. One substrate is removed from each of the sample Bragg gratings and the gratings are scanned in the Cary500. The nominal notch wavelength is 536 nm. After liquid crystal removal, the sample Bragg gratings are then split into four groups for further treatment: (A) anti-cortisol attachment with subsequent incubation in cortisol; (B) anti-cortisol attachment with no subsequent incubation in cortisol; (C) no antibody attachment with subsequent incubation in cortisol; and (D) anti-cortisol attachment with subsequent incubation in Gliadin. Two sample Bragg gratings from each group (A) – (D) are subjected to the entire cortisol test, consisting of attachment, incubation, spectrometer measurements of diffraction notch, and color test. The

remaining two sample Bragg gratings from groups (A) – (D) are run only through the diffraction notch test.

[0111] Sample Bragg gratings in groups (A), (B), and (D) are subjected to the same attachment procedure, while no antibody (anti-cortisol) is added to the sample Bragg gratings in group (C). At the conclusion of these procedures, all of the sample Bragg gratings are measured using the Cary500. In all samples, the diffraction notch red shifts approximately 6% due to the increased refractive index as mass, i.e., anti-cortisol and/or casein, was added to the vacant pores.

[0112] Sample Bragg gratings in groups (A), (C), and (D) are then incubated in cortisol, and all sample Bragg gratings are re-measured with the Cary500. Only sample Bragg gratings in group (A) (the only group treated with anti-cortisol attachment and cortisol incubation) exhibit a red shift (approximately 1%), indicating the binding of cortisol to anti-cortisol thereby increases the mass in the pores and thus increasing the refractive index. **Fig. 23** exemplifies this shift when an anti-cortisol activated grating **2300** is incubated in cortisol **2310**.

[0113] Two sample Bragg gratings from groups (A), (B), and (C) are subjected to a color test verification. The color test consists of a competitive reaction between the antigen, i.e., cortisol, and an enzyme-conjugated antigen, i.e., anti-cortisol. Sample Bragg gratings in groups (A) and (C) are incubated in equal volumes of the antigen and the enzyme-conjugated antigen, while group (B) sample Bragg gratings are incubated in the enzyme-conjugated antigen only. Antigen and enzyme-conjugated antigen molecules bind with present antibodies in proportion to their relative concentration. When the chromogenic solution containing TMB is added, TMB reacts with the enzyme-conjugated antigen, inducing a color change. HCl is again added to stop the reaction and stabilize the sample Bragg gratings for subsequent spectrometer runs. Hence, sample Bragg gratings with a higher proportion of enzyme-conjugated antigens exhibit a stronger color change, i.e., have a higher absorbance at 450 nm. Thus, the absorbance at 450 nm is inversely proportional to the concentration of antigen, i.e., cortisol, present, as exemplified in the test results in **Fig. 24**. The highest absorbance is for a sample **2400** not incubated in cortisol because only enzyme-conjugated antigen binds to the present antibodies. Sample Bragg grating **2410** incubated in cortisol exhibits a lower absorbance, i.e., some of the antibody sites are bound with cortisol and some with enzyme-conjugated antigen. Sample Bragg grating **2420** that was

not treated with antibody attachment shows the least absorbance, i.e., there are no sites for the enzyme-conjugated antigen to bind to and hence produces a color change.

[0114] The embodiments described herein are intended to be exemplary, and while including and describing the best mode of practicing, are not intended to limit the invention. Those skilled in the art appreciate the multiple variations to the embodiments described herein which fall within the scope of the invention.